ESPP2 H-151 Identification of a Small Non-Coding RNA in *Desulfovibrio vulgaris*

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Environmental Stress Pathway Project

DOE GENOMICS:GTL ACCELERATING DISCOVERY FOR ENERGY AND ENVIRONMENT — OFFICE OF SCIENCE U.S. DEPARTMENT OF ENERGY

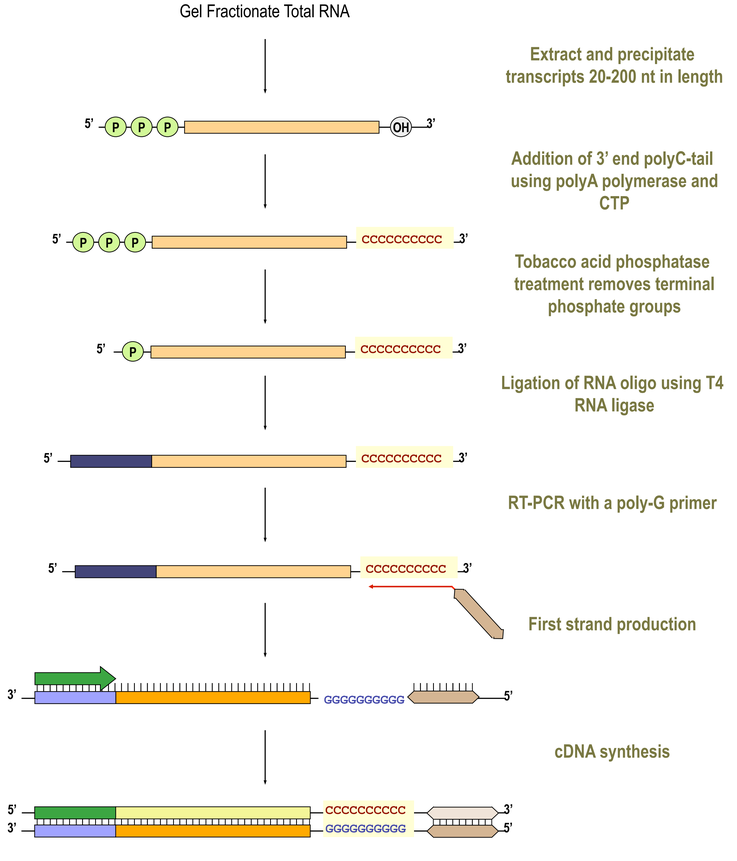
VIMSS Virtual Institute for Microbial Stress and Survival | Massachusetts Institute of Technology | University of Missouri | University of Washington | Montana State University | Southern Illinois University Carbondale | University of Oklahoma | Sandia National Laboratories | Oak Ridge National Laboratory | Berkeley University of California | 75 Years of World Class Science | http://vimss.lbl.gov/

ABSTRACT

Identification and analysis of small non-coding RNAs (sRNAs) in *D. vulgaris* and other metal-reducing bacteria are essential for uncovering novel regulatory mechanisms involved in processes critical to the DOE such as stress response, environmental adaptation, and contaminant remediation. Previous work by the 'Environmental Stress Pathway Project' has enhanced our systems knowledge of *D. vulgaris* via valuable transcriptional and proteomic profiles, however regulation by sRNAs could not be detected in those experiments. In an effort to identify sRNAs in *D. vulgaris*, a strategy for cloning total RNA ranging in size from 20-200 nt was employed. Following addition of directional aptamer sequences, cDNAs were produced and cloned for sequencing. Sequence analysis of a small portion of the resulting cDNA library yielded two identical ~65 nt sRNA clones (Dv-sRNA2) possessing complementary sequence to the RBS of open reading frame (ORF) DVU0678. While DVU0678 is adjacent to the Dv-sRNA2 gene, the ORF is transcribed from the opposite chromosomal strand. Northern analysis specialized for sRNAs verified the expression of Dv-sRNA2 as an individual transcript under anaerobic lactate/sulfate growth (LS4D medium). These data suggest that when Dv-sRNA2 is transcribed, translation of DVU0678 will be inhibited. DVU0678 has been annotated to encode a putative 34 amino acid protein unique to *D. vulgaris* strains Hildenborough and DP4, hampering our abilities to discern the role of DVU0678 in the cell. Further sequence analysis of the Dv-sRNA DNA locus by 'PromScan' identified a putative sigma^{54}-recognition site (97% probability) 43 nt upstream of the predicted sRNA transcriptional start site and therefore suggests that Dv-sRNA2 may be member of the sigma^{54} regulon. A perfect stem-loop terminator was also identified 26 nt downstream of the Dv-sRNA2 DNA sequence. Current analysis is underway to ascertain the expression profile for this sRNA as well as the effect over -expression has on the physiology and transcriptional response of *D. vulgaris* under multiple environmental conditions.

MATERIALS AND METHODS

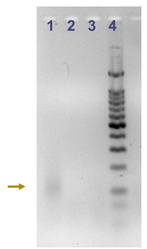
Cloning of Small RNA Fraction



RESULTS

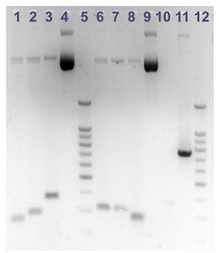
Cloning and Sequencing of Small RNAs

cDNA for library



1: cDNA
2: No template
3: No reverse transcriptrase
4: 100 bp ladder

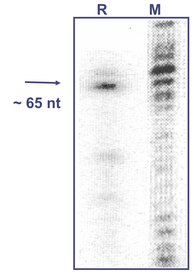
PCR verification of sRNA clone inserts



1-3: library clones
4: plasmid only
5: 100 bp ladder
6-8: library clones
9: plasmid only
10: neg. control
11: primer positive control
12: 100 bp ladder

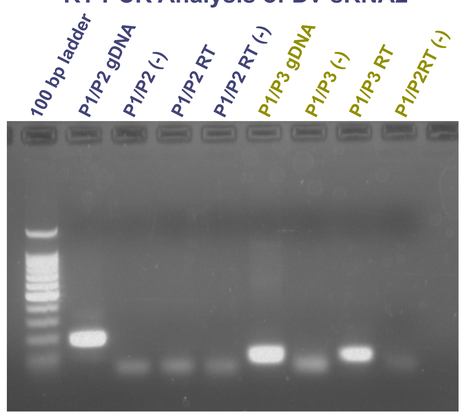
Expression of Dv-sRNA2

Northern Verification of Dv-sRNA2 Expression



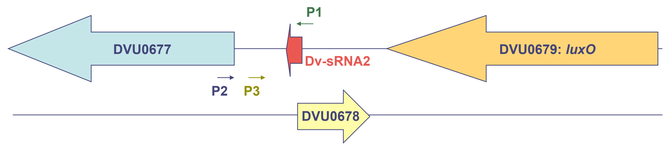
R: 10 µg total RNA
M: Marker

RT-PCR Analysis of Dv-sRNA2

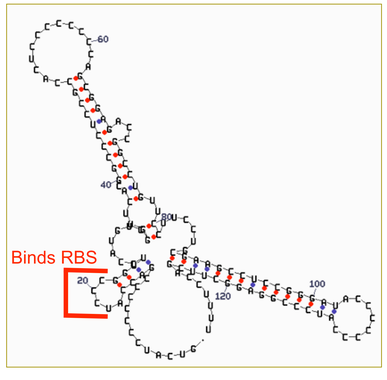


Features of Dv-sRNA2

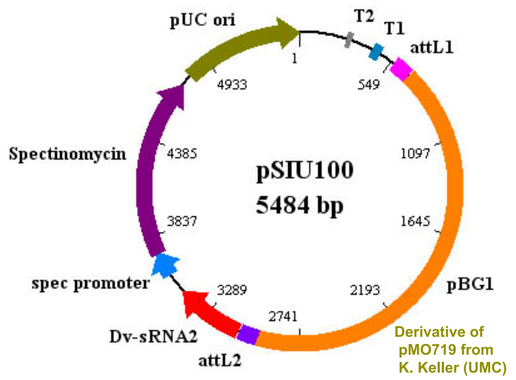
- **Antisense to RBS of DVU0678 which has been annotated as a putative protein.**
- **PromScan identified a putative sigma^{54} recognition site 43 nt upstream with 97% probability.**
- **A perfect stem-loop terminator is located 26 nt downstream of the predicted transcript.**



MFOLD Secondary Structure Prediction of Dv-sRNA2

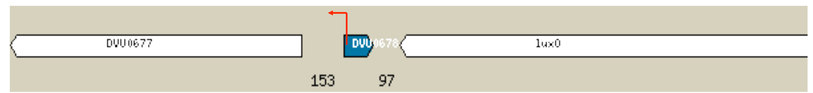


Vector constructed to overexpress Dv-sRNA2



SUMMARY

61 nt target starts 37 nt into the 5' end of DVU0678



DVU0678 message: 34 AA hypothetical protein

RBS of DVU0678 binding site blocked if small RNA 2A present: **No translation.**

CONCLUSIONS

- In addition to computational searches, random cloning of the small RNA fraction can be used to identify unknown sRNAs.
- The cloning method does not discriminate between degraded mRNAs and non-coding RNAs.
- Dv-sRNA2 is a ~65 nt sRNA in *D. vulgaris* that is expressed under normal growth conditions.
- Dv-sRNA2 is anti-sense to the RBS of DVU0678, an ORF which encodes a putative protein.
- It is predicted that when Dv-sRNA2 is expressed, DVU0678 will not be translated based on the inability of the ribosome to bind.
- Sequence analysis identified a putative sigma^{54}-binding site with 97% probability (PromScan) 43 bp upstream of the Dv-sRNA2 gene and a perfect rho-independent terminator 26 bp downstream.
- RT-PCR confirmed that Dv-sRNA2 is not part of a 5' untranslated region of the DVU0677 ORF.
- To ascertain the cellular role of Dv-sRNA2, phenotypic analysis of an overexpression strain (KB100) is underway. Northern analysis under various stress conditions is also on-going to determine the expression pattern of Dv-sRNA2.

ACKNOWLEDGEMENT

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